TABLE 3. Electromorph frequencies and variability estimates for polymorphic loci in 38 populations of Japanese *Cynops*

Locus	C. pyrrhogaster									
	Sasayama race								Hiroshima race	
	1	2	3	4	5	6	7	8	9	10
Acp-A	c	c	c	c	c	c	c	c	c	c
S-Aat-A	b	a(.069) b(.914) d(.017)	a(.107) b(.893)	a(.125) b(.875)	b	b(.974) d(.026)	b	b	a(.139) b(.861)	b
Est-1	b	b	b	b	b	b(.947) c(.053)	b(.947) c(.053)	b	b	b
Gpi-A	c(.975) d(.025)	b(.013) c(.884) d(.103)	b(.063) c(.906) d(.031)	b(.125) c(.750) d(.125)	c	c	c	c	c	a(.150) c(.750) c(.100)
Iddh-A	b	b	b	b	b	b	b	b	b	b
Ldh-A	b	b	b	b	b	b	b	b	b	b
Ldh-B	c	c	c	c	a(.125) c(.875)	a(.233) c(.767)	a(.175) c(.825)	a(.063) c(.937)	c	c
M-Mdh-A	b	b	b	b	b	b	b	b	b	b
S-Mdh-A	c	b(.016) c(.968) d(.016)	c(.937) d(.063)	c(.937) e(.063)	c(.875) d(.125)	a(.026) c(.974)	a(.025) c(.725) d(.250)	c(.438) d(.562)	c	c
M-Me-A	b(.941) c(.059)	b(.677) c(.323)	b(.438) c(.562)	b(.375) c(.625)	b(.375) c(.625)	b(.895) c(.105)	b(.667) c(.333)	b(.875) c(.125)	b(.643) c(.357)	b(.333) c(.667)
S-Me-A	b	a(.219) b(.781)	b	b	b	b	b(.950) c(.050)	b(.750) c(.250)	b(.850) c(.150)	b
Pgm-A	a(.025) b(.500) d(.475)	b(.516) d(.484)	a(.031) b(.375) d(.594)	b(.375) d(.625)	b(.250) d(.750)	a(.053) b(.342) d(.605)	b(.450) d(.550)	b(.313) d(.687)	a(.025) b(.950) d(.025)	a(.150) b(.775) d(.075)
Pgdh-A	b	b	b	b	b	b(.972) c(.028)	a(.025) b(.975)	b b	b(.900) c(.100)	b
S-Sod-A	b	b	b	b	b	b	b	b	b	a(.150) b(.850)
% loci polymorphic	20.0	40.0	33.3	33.3	26.7	53.3	46.7	33.3	33.3	26.7
number of alleles per locus	1.27	1.60	1.47	1.40	1.27	1.60	1.53	1.33	1.40	1.40
Mean heterozygosity	.041	.083	.106	.108	.117	.113	.093	.075	.060	.085

Table 3. Continued

| Locus | C. pyrrhogaster | | | | | | | | | |
| | Hiroshima race | | | | | | | | | |
	11	12	13	14	15	16	17	18	19	20
Acp-A	c	c	c	c	c	c	c	c	c	c
S-Aat-A	b	b(.853) c(.147)	a(.050) b(.950)	b(.950) e(.050)	b	b(.775) a(.225)	b	a(.025) b(.975)	b(.944) d(.056)	b(.950) d(.050)
Est-1	a(.025) b(.975)	a(.075) b(.925)	a(.250) b(.750)	b	b(.800) c(.200)	a(.575) b(.425)	a(.306) b(.694)	a(.100) b(.900)	a(.250) b(.750)	b
Gpi-A	a(.100) c(.850) d(.050)	c(.975) d(.025)	b(.025) c(.900) d(.075)	c(.955) d(.045)	c	c	c(.972) d(.028)	b(.425) c(.575)	b(.025) c(.975)	c(.975) d(.025)
Iddh-A	b	b	b	b	b	b	b	b	b	b
Ldh-A	b	a(.075) b(.925)	b	b	b	b	b	b	b	b
Ldh-B	c	c	b(.075) c(.925)	c	c	c	c	c	c	c
M-Mdh-A	b	b	b	a(.050) b(.950)	b	b	b	b	b	b
S-Mdh-A	c	c	c	b(.048) c(.857) d(.095)	b(.300) c(.700)	b(.050) c(.950)	c	b(.025) c(.975)	b(.026) c(.974)	c(.974) d(.026)
M-Me-A	b(.684) c(.316)	b(.472) c(.528)	b(.769) c(.231)	b(.735) c(.265)	b(.500) c(.500)	b(.529) c(.471)	b	b(.971) c(.029)	b(.393) c(.607)	b(.154) c(.846)
S-Me-A	b(.975) c(.025)	b	b	a(.045) b(.955)	b	b(.800) c(.200)	b	b	a(.025) b(.950) c(.025)	b
Pgm-A	a(.050) b(.800) d(.075) e(.075)	b	a(.075) b(.925)	a(.045) b(.955)	b(.800) d(.200)	b	b	a(.025) b(.975)	a(.300) b(.700)	a(.100) b(.900)
Pgdh-A	b	a(.025) b(.950) c(.025)	a(.075) b(.925)	b	a(.100) b(.900)	b	a(.118) b(.882)	a(.125) b(.875)	b	b(.950) c(.050)
S-Sod-A	b(.950) c(.050)	b(.975) c(.025)	b(.750) c(.250)	b	b	b(.725) c(.275)	b(.750) c(.250)	b(.950) c(.050)	b(.900) c(.100)	b
% loci polymorphic	40.0	46.7	53.3	46.7	33.3	40.0	26.7	53.3	53.3	40.0
number of alleles per locus	1.60	1.53	1.60	1.53	1.33	1.40	1.27	1.53	1.60	1.40
mean heterozygosity	.061	.049	.090	.042	.129	.097	.068	.074	.091	.027

TABLE 3. Continued

Locus	C. pyrrhogaster Hiroshima race			C. ensicauda Amami Group				C. ensicauda Okinawa Group	
	21	22	23	24	25	26	27	28	29
Acp-A	c	c	c	a	a	a(.917) b(.083)	a	a	a
S-Aat-A	b(.722) d(.278)	b(.357) d(.643)	a(.056) b(.500) d(.444)	a(.058) b(.942)	a(.050) b(.900) d(.050)	b(.944) d(.056)	a(.025) b(.975)	a(.100) b(.900)	a(.125) b(.687) d(.188)
Est-1	b	b	b	b	b	b	b	b	b
Gpi-A	b(.971) c(.029)	b(.955) c(.045)	b(.333) c(.667)	c	c	c	b(.029) c(.971)	c	b(.125) c(.875)
Iddh-A	b	b	b	a	a	a	a	a	a
Ldh-A	b	b	b	b	b	b	b	b	b
Ldh-B	c	c	c	b(.096) c(.904)	b(.275) c(.725)	c	c	c	c
M-Mdh-A	b	b	b	b	b	b	b	b	b
S-Mdh-A	c	c	b(.028) c(.972)	d	d(.921) e(.079)	d	d	c(.800) d(.200)	c(.438) d(.562)
M-Me-A	b(.500) c(.500)	c	b(.429) c(.571)	a	a	a	a	a	a
S-Me-A	b	b	b	b	b(.875) c(.125)	b	b	b(.300) c(.700)	b(.313) c(.687)
Pgm-A	a(.500) b(.500)	a(.727) b(.273)	a(.222) b(.750) c(.028)	b(.944) d(.037) e(.019)	b	b(.944) f(.056)	b	b(.625) d(.375)	b
Pgdh-A	b	b	b	a(.042) b(.937) c(.021)	a(.025) b(.975)	a(.333) b(.667)	a(.350) b(.650)	b	b
S-Sod-A	c	c	b(.361) c(.639)	b	b	b	b	b	b
% loci polymorphic	26.7	20.0	40.0	26.7	33.3	28.6	20.0	28.6	28.6
number of alleles per locus	1.27	1.20	1.53	1.40	1.40	1.29	1.20	1.29	1.36
mean heterozygosity	.147	.040	.152	.031	.054	.060	.044	.111	.098

Table 3. Continued

Locus	C. ensicauda								
	Okinawa Group								
	30	31	32	33	34	35	36	37	38
Acp-A	a	a	a	a	a	a	a	a	a
S-Aat-A	a(.100) b(.800) d(.100)	b(.900) d(.100)	b	b(.972) d(.028)	a(.056) b(.944)	a(.100) b(.900)	b(.750) d(.250)	a(.037) b(.815) d(.148)	b(.833) d(.167)
Est-1	b	b	b	a(.028) b(.944) c(.028)	b	b	b	b	b
Gpi-A	c	b(.050) c(.900) d(.050)	c	c(.861) d(.139)	c	c	b(.125) c(.875)	b(.033) c(.967)	c
Iddh-A	a	a	a	a	a	a	a	a	a
Ldh-A	b	b	b	b	b	b	b	b	b
Ldh-B	c	c	c	c	c	c	c	c	c
M-Mdh-A	b	b	b	b	b	b	b	b	b
S-Mdh-A	c(.500) d(.500)	c(.850) d(.150)	c(.550) d(.450)	c(.719) d(.281)	c(.556) d(.444)	c(.600) d(.350) e(.050)	b(.333) c(.667)	c(.696) d(.304)	c(.550) d(.450)
M-Me-A	a	a	a	a	a	a	a	a	a
S-Me-A	b(.100) c(.900)	b(.450) c(.550)	b(.300) c(.700)	b(.306) c(.694)	b(.278) c(.722)	b(.300) c(.700)	b(.125) c(.875)	b(.117) c(.883)	b(.400) c(.600)
Pgm-A	b	b	b	b	b	b(.950) d(.050)	b	b	b(.950) d(.050)
Pgdh-A	b	b	b	b(.972) c(.028)	b	b	b	b(.981) c(.019)	b
S-Sod-A	b	b	b	b	b	b	b	b	b
% loci polymorphic	21.4	26.7	13.3	40.0	28.6	21.4	28.6	35.7	26.7
number of alleles per locus	1.29	1.33	1.13	1.47	1.36	1.21	1.29	1.43	1.27
mean heterozygosity	.086	.053	.047	.081	.079	.079	.119	.073	.096

TABLE 4. Probability levels of electromorph frequency heterogeneity with a contingency Chi-square test

Locus	Total	C. Pyrrhogaster	C. ensicauda		
			Total	Amami	Okinawa
Acp-A	.001	—	.05	.05	—
S-Aat-A	.001	.001	NS	NS	.05
Est-1	.001	.001	NS	—	NS
Gpi-A	.001	.001	.001	NS	.01
Iddh-A	.001	—	—	—	—
Ldh-A	.001	.001	—	—	—
Ldh-B	.001	.001	.001	.001	—
M-Mdh-A	NS	.05	—	—	—
S-Mdh-A	.001	.001	.001	.05	.001
M-Me-A	.001	.001	—	—	—
S-Me-A	.001	.001	.001	.01	NS
Pgm-A	.001	.001	.001	NS	.001
Pgdh-A	.001	.001	.001	.001	NS
S-Sod-A	.001	.001	—	—	—

NS indicates statistically insignificant difference at P<0.05.
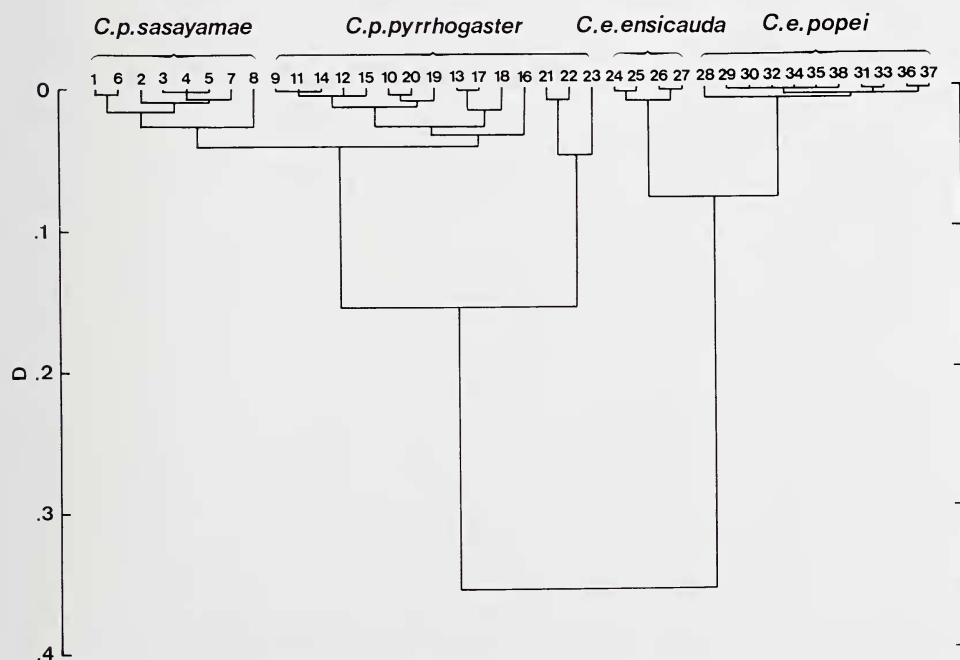
FIG. 2. UPGMA phenogram of populations of Japanese *Cynops* studied. Scale is in genetic distance units of Nei (1978).

T. Hayashi and M. Matsui

Table 5. Nei's genetic similarity (below diagonal) and distance (above diagonal) between populations of Japanese *Cynops* studied

| | C. pyrrhogaster | | | | | | | | | |
| | Sasayama race | | | | | | | | Hiroshima race | |
	1	2	3	4	5	6	7	8	9	10
1		0.007	0.017	0.024	0.023	0.004	0.010	0.026	0.022	0.038
2	0.993		0.006	0.008	0.011	0.012	0.009	0.029	0.017	0.024
3	0.983	0.994		−0.005	−0.005	0.017	0.007	0.034	0.026	0.018
4	0.977	0.992	1.005		−0.004	0.024	0.011	0.040	0.031	0.017
5	0.977	0.989	1.005	1.004		0.015	0.003	0.028	0.041	0.028
6	0.996	0.988	0.983	0.976	0.985		0.006	0.025	0.035	0.049
7	0.990	0.991	0.993	0.989	0.997	0.994		0.010	0.026	0.032
8	0.974	0.971	0.967	0.961	0.973	0.976	0.990		0.057	0.076
9	0.978	0.984	0.974	0.969	0.960	0.966	0.974	0.944		0.014
10	0.962	0.977	0.982	0.983	0.973	0.952	0.969	0.927	0.986	
11	0.987	0.988	0.980	0.977	0.969	0.975	0.981	0.951	0.997	0.991
12	0.967	0.976	0.973	0.970	0.961	0.954	0.968	0.927	0.997	0.992
13	0.976	0.973	0.961	0.956	0.946	0.963	0.966	0.933	0.990	0.977
14	0.982	0.983	0.970	0.965	0.958	0.968	0.977	0.952	0.998	0.983
15	0.980	0.987	0.988	0.984	0.982	0.972	0.989	0.957	0.994	0.991
16	0.937	0.946	0.938	0.934	0.925	0.925	0.937	0.903	0.969	0.958
17	0.973	0.960	0.939	0.931	0.924	0.957	0.954	0.929	0.979	0.953
18	0.972	0.963	0.945	0.943	0.925	0.955	0.953	0.928	0.978	0.958
19	0.963	0.974	0.978	0.974	0.969	0.955	0.969	0.929	0.986	0.992
20	0.944	0.964	0.974	0.973	0.966	0.934	0.958	0.911	0.983	0.995
21	0.844	0.854	0.860	0.870	0.839	0.833	0.836	0.802	0.853	0.884
22	0.750	0.778	0.805	0.819	0.788	0.746	0.760	0.715	0.775	0.828
23	0.919	0.932	0.936	0.938	0.919	0.908	0.918	0.879	0.945	0.956
24	0.712	0.714	0.719	0.713	0.718	0.698	0.741	0.748	0.732	0.724
25	0.704	0.707	0.709	0.703	0.709	0.691	0.734	0.740	0.729	0.717
26	0.685	0.685	0.690	0.684	0.689	0.668	0.715	0.721	0.709	0.698
27	0.701	0.702	0.707	0.701	0.705	0.685	0.729	0.735	0.725	0.715
28	0.736	0.747	0.742	0.737	0.740	0.724	0.741	0.741	0.751	0.731
29	0.688	0.702	0.692	0.688	0.681	0.667	0.702	0.711	0.732	0.704
30	0.678	0.693	0.680	0.673	0.671	0.658	0.692	0.705	0.725	0.692
31	0.752	0.762	0.752	0.748	0.741	0.734	0.752	0.737	0.787	0.769
32	0.729	0.739	0.729	0.723	0.723	0.711	0.740	0.744	0.765	0.743
33	0.733	0.745	0.733	0.729	0.723	0.715	0.738	0.733	0.770	0.751
34	0.713	0.724	0.714	0.708	0.706	0.694	0.722	0.723	0.751	0.725
35	0.705	0.717	0.706	0.699	0.698	0.685	0.717	0.721	0.746	0.720
36	0.689	0.704	0.688	0.683	0.675	0.668	0.688	0.684	0.735	0.705
37	0.690	0.704	0.690	0.684	0.679	0.670	0.696	0.698	0.736	0.705
38	0.735	0.745	0.737	0.732	0.731	0.718	0.746	0.748	0.768	0.747

TABLE 5. Continued

	C. pyrrhogaster									
	Hiroshima race									
	11	12	13	14	15	16	17	18	19	20
1	0.013	0.034	0.024	0.018	0.020	0.065	0.028	0.029	0.037	0.058
2	0.012	0.025	0.027	0.018	0.013	0.056	0.040	0.037	0.026	0.037
3	0.020	0.027	0.040	0.030	0.012	0.064	0.062	0.057	0.023	0.027
4	0.023	0.030	0.045	0.036	0.016	0.069	0.071	0.058	0.026	0.027
5	0.032	0.040	0.055	0.043	0.018	0.078	0.079	0.078	0.032	0.034
6	0.025	0.047	0.037	0.033	0.028	0.078	0.044	0.046	0.046	0.068
7	0.019	0.033	0.035	0.023	0.011	0.065	0.047	0.048	0.032	0.043
8	0.051	0.075	0.069	0.049	0.043	0.102	0.074	0.075	0.073	0.093
9	0.003	0.003	0.010	0.002	0.006	0.031	0.022	0.023	0.014	0.018
10	0.009	0.008	0.023	0.017	0.009	0.043	0.048	0.042	0.008	0.005
11		0.006	0.006	0.002	0.005	0.035	0.018	0.017	0.012	0.021
12	0.994		0.012	0.007	0.004	0.027	0.028	0.031	0.009	0.008
13	0.994	0.988		0.009	0.014	0.017	0.002	0.015	0.014	0.035
14	0.998	0.993	0.991		0.004	0.037	0.017	0.017	0.017	0.024
15	0.995	0.996	0.986	0.996		0.030	0.028	0.031	0.007	0.010
16	0.966	0.974	0.983	0.964	0.970		0.026	0.054	0.019	0.043
17	0.983	0.972	0.998	0.983	0.972	0.974		0.016	0.034	0.064
18	0.983	0.969	0.985	0.983	0.970	0.947	0.984		0.043	0.062
19	0.989	0.991	0.986	0.983	0.993	0.981	0.966	0.958		0.009
20	0.980	0.992	0.965	0.976	0.990	0.958	0.938	0.940	0.991	
21	0.874	0.858	0.886	0.854	0.849	0.861	0.861	0.891	0.880	0.856
22	0.791	0.791	0.795	0.768	0.779	0.797	0.753	0.784	0.826	0.815
23	0.949	0.951	0.956	0.941	0.941	0.947	0.933	0.935	0.957	0.947
24	0.730	0.733	0.716	0.747	0.747	0.695	0.707	0.707	0.721	0.734
25	0.724	0.728	0.712	0.742	0.740	0.694	0.701	0.701	0.715	0.729
26	0.705	0.710	0.692	0.724	0.727	0.667	0.686	0.686	0.695	0.711
27	0.721	0.726	0.710	0.739	0.742	0.687	0.704	0.706	0.712	0.727
28	0.740	0.734	0.715	0.738	0.739	0.711	0.704	0.703	0.730	0.734
29	0.713	0.717	0.694	0.725	0.715	0.698	0.684	0.692	0.703	0.715
30	0.703	0.705	0.683	0.714	0.704	0.692	0.675	0.674	0.694	0.704
31	0.776	0.778	0.760	0.779	0.771	0.755	0.750	0.753	0.766	0.775
32	0.752	0.753	0.734	0.760	0.753	0.733	0.726	0.725	0.743	0.751
33	0.758	0.758	0.742	0.763	0.755	0.741	0.733	0.734	0.748	0.756
34	0.734	0.736	0.715	0.742	0.735	0.714	0.706	0.705	0.725	0.734
35	0.729	0.731	0.711	0.739	0.730	0.710	0.702	0.701	0.720	0.730
36	0.715	0.717	0.694	0.721	0.718	0.706	0.685	0.693	0.706	0.714
37	0.715	0.717	0.695	0.722	0.711	0.704	0.687	0.688	0.706	0.715
38	0.755	0.757	0.738	0.764	0.758	0.738	0.729	0.728	0.747	0.756

Table 5. Continued

| | *C. pyrrhogaster* | | | *C. ensicauda* | | | | | | |
| | Hiroshima race | | | Amami Group | | | Okinawa Group | | | |
	21	22	23	24	25	26	27	28	29	30
1	0.169	0.287	0.084	0.339	0.351	0.378	0.355	0.307	0.374	0.388
2	0.158	0.251	0.071	0.337	0.347	0.378	0.353	0.291	0.354	0.366
3	0.151	0.217	0.066	0.330	0.344	0.370	0.347	0.298	0.368	0.386
4	0.139	0.200	0.064	0.338	0.352	0.380	0.355	0.305	0.374	0.396
5	0.176	0.238	0.085	0.331	0.344	0.372	0.350	0.302	0.384	0.399
6	0.183	0.293	0.096	0.359	0.369	0.404	0.379	0.323	0.404	0.418
7	0.179	0.274	0.085	0.300	0.310	0.336	0.316	0.299	0.353	0.368
8	0.220	0.336	0.129	0.291	0.301	0.327	0.309	0.300	0.341	0.350
9	0.159	0.255	0.056	0.312	0.316	0.344	0.321	0.286	0.312	0.322
10	0.123	0.189	0.045	0.323	0.332	0.359	0.335	0.313	0.351	0.368
11	0.135	0.235	0.052	0.315	0.323	0.350	0.327	0.301	0.338	0.353
12	0.153	0.234	0.051	0.310	0.317	0.342	0.320	0.309	0.333	0.349
13	0.121	0.229	0.045	0.333	0.340	0.369	0.342	0.336	0.365	0.381
14	0.157	0.264	0.061	0.291	0.298	0.323	0.302	0.303	0.321	0.337
15	0.163	0.249	0.061	0.292	0.301	0.319	0.299	0.303	0.336	0.352
16	0.150	0.227	0.054	0.363	0.365	0.405	0.376	0.341	0.359	0.368
17	0.150	0.284	0.070	0.346	0.355	0.377	0.350	0.351	0.379	0.393
18	0.115	0.243	0.067	0.346	0.355	0.377	0.348	0.352	0.368	0.394
19	0.128	0.191	0.044	0.327	0.335	0.364	0.340	0.315	0.352	0.366
20	0.155	0.205	0.054	0.309	0.317	0.341	0.319	0.309	0.335	0.351
21		0.009	0.027	0.537	0.551	0.604	0.549	0.544	0.558	0.613
22	0.991		0.069	0.644	0.658	0.723	0.660	0.650	0.657	0.724
23	0.974	0.934		0.397	0.405	0.443	0.411	0.399	0.408	0.444
24	0.585	0.525	0.672		0.003	0.005	0.006	0.093	0.054	0.081
25	0.576	0.518	0.667	0.997		0.013	0.013	0.085	0.043	0.067
26	0.547	0.485	0.642	0.995	0.987		−0.002	0.104	0.062	0.090
27	0.578	0.517	0.663	0.994	0.987	1.002		0.106	0.063	0.091
28	0.580	0.522	0.671	0.911	0.919	0.901	0.900		0.015	0.011
29	0.572	0.518	0.665	0.948	0.958	0.940	0.939	0.985		−0.003
30	0.542	0.485	0.642	0.922	0.936	0.914	0.913	0.989	1.003	
31	0.638	0.578	0.722	0.928	0.936	0.915	0.921	0.995	0.989	0.986
32	0.600	0.537	0.687	0.945	0.900	0.876	0.876	0.989	0.990	0.995
33	0.613	0.550	0.696	0.929	0.917	0.896	0.895	0.992	0.995	1.002
34	0.571	0.507	0.667	0.935	0.960	0.942	0.944	0.992	1.001	0.998
35	0.565	0.500	0.661	0.939	0.949	0.931	0.931	0.993	1.000	1.003
36	0.575	0.521	0.666	0.884	0.945	0.926	0.926	0.998	0.999	1.001
37	0.561	0.503	0.657	0.903	0.954	0.934	0.938	0.992	0.999	1.001
38	0.609	0.555	0.702	0.952	0.938	0.916	0.922	0.994	0.993	0.995

TABLE 5. Continued

| | C. ensicauda | | | | | | | |
| | Okinawa Group | | | | | | | |
	31	32	33	34	35	36	37	38
1	0.285	0.316	0.310	0.338	0.349	0.373	0.371	0.308
2	0.272	0.302	0.294	0.323	0.333	0.351	0.350	0.294
3	0.285	0.316	0.311	0.337	0.349	0.374	0.372	0.305
4	0.291	0.324	0.316	0.346	0.358	0.381	0.380	0.313
5	0.300	0.325	0.324	0.348	0.360	0.394	0.387	0.314
6	0.309	0.341	0.336	0.366	0.378	0.403	0.401	0.331
7	0.285	0.300	0.304	0.326	0.333	0.373	0.362	0.292
8	0.305	0.296	0.310	0.325	0.328	0.380	0.359	0.291
9	0.239	0.267	0.261	0.286	0.293	0.308	0.307	0.263
10	0.262	0.297	0.287	0.321	0.328	0.349	0.350	0.292
11	0.253	0.286	0.277	0.309	0.315	0.336	0.336	0.281
12	0.251	0.284	0.277	0.307	0.313	0.333	0.333	0.278
13	0.275	0.309	0.299	0.336	0.342	0.365	0.363	0.304
14	0.249	0.274	0.271	0.298	0.302	0.327	0.326	0.269
15	0.260	0.283	0.281	0.308	0.314	0.331	0.341	0.278
16	0.280	0.310	0.300	0.337	0.342	0.348	0.351	0.303
17	0.287	0.320	0.311	0.348	0.353	0.378	0.375	0.316
18	0.284	0.321	0.309	0.350	0.355	0.367	0.374	0.317
19	0.266	0.298	0.290	0.322	0.329	0.349	0.349	0.291
20	0.256	0.286	0.280	0.309	0.315	0.337	0.336	0.280
21	0.450	0.511	0.489	0.561	0.570	0.554	0.579	0.496
22	0.547	0.622	0.597	0.678	0.693	0.652	0.686	0.589
23	0.326	0.375	0.363	0.405	0.414	0.406	0.421	0.354
24	0.075	0.056	0.074	0.067	0.063	0.123	0.102	0.049
25	0.066	0.047	0.064	0.057	0.053	0.105	0.087	0.041
26	0.088	0.068	0.088	0.076	0.071	0.132	0.110	0.060
27	0.083	0.064	0.082	0.077	0.072	0.133	0.111	0.057
28	0.005	0.008	0.006	0.002	0.007	0.011	0.008	0.008
29	0.011	0.001	0.007	0.001	0.000	0.011	0.005	−0.001
30	0.014	−0.001	0.005	−0.001	−0.003	0.005	−0.002	0.002
31		0.005	0.000	0.003	0.006	0.007	0.008	0.004
32	0.995		0.001	−0.003	−0.004	0.011	0.004	0.000
33	1.000	0.999		−0.001	0.000	0.006	0.004	0.003
34	0.997	1.003	1.001		−0.004	0.007	0.002	−0.001
35	0.994	1.004	1.000	1.004		0.009	0.002	−0.001
36	0.993	0.989	0.994	0.994	0.991		0.000	0.012
37	0.992	0.996	0.996	0.998	0.998	1.000		0.006
38	0.996	1.000	0.997	1.001	1.001	0.989	0.994	

the mean D value between *C. ensicauda* and 20 remaining populations of *C. pyrrhogaster* is 0.330 (range 0.239–0.418). Thus, in spite of their adjacency to the range of *C. ensicauda*, southernmost populations of *C. pyrrhogaster* reach higher level of genetic differentiation from *C. ensicauda* than do remaining conspecific populations.

DISCUSSION

No overlap of electromorphs was detected at three loci (*Acp-A*, *Iddh-A* and *S-Me-A*) between *C. ensicauda* and *C. pyrrhogaster*, and no individual showed any intermediate condition. Furthermore in specimens of *C. pyrrhogaster* from northeastern Japan, we have not detected any individual sharing any electromorph with *C. ensicauda* at the three loci (Hayashi and Matsui, unpublished). Therefore, it is clear that *C. ensicauda* and *C. pyrrhogaster* are genetically distinct from each other.

Levels of genetic differentiation have been investigated at the interspecific and intersubspecific ranks in other genera of the family Salamandridae (*Taricha* [6] and *Triturus* [22]). Genetic differentiation estimates derived from these data might provide an indication of the range in values one might expect between biological species within this family. Nei's D values identified between the two species of Japanese *Cynops* have the range of 0.239 to 0.724 ($\bar{x}=0.356$) and are smaller than interspecific values calculated in *Triturus* species (range from 0.702 to 1.321, $\bar{x}=1.117$), but nearly correspond with the values estimated for three species of *Taricha* (range from 0.261 to 0.687, $\bar{x}=0.466$). It is also noteworthy that the greatest genetic difference was observed between geographically most adjacent populations of *C. pyrrhogaster* from southernmost Kyushu and *C. ensicauda*. From these genetic view points, two forms of Japanese *Cynops*, one from the main islands (*pyrrhogaster*) and another from the Ryukyu Archipelago (*ensicauda*), are judged to be well differentiated from each other at the species level, and the designation of the latter form as a subspecies of the former is unfounded.

Inger [1] studied morphological variations in newts from Okinawajima and Amami-Oshima Is-lands, and considered the differences between these two populations sufficient to warrant subspecific distinction. Thus he described the Okinawa population as a distinct subspecies, *Triturus ensicaudus popei*. On the contrary, Koba [23] and Nakamura and Uéno [4], without presenting valid evidence, opposed such a distinction. Our biochemical analysis shows that populations from the Amami Group form a group distinct from those from Okinawa Group with Nei's D values between them ranging from 0.059 to 0.140 ($\bar{x}=0.084$). Although the amount of genetic differentiation between these two groups is smaller than values estimated among intersubspecific populations in *Taricha* (range 0.104–0.309) [24], the geographic pattern of biochemical variation is consistent with the pattern of morphological variations reported by Inger [1], and seems to support the subspecific status of *C. ensicauda popei* (new comb.).

Sawada [2] divided *C. pyrrhogaster* into six "local races" (Hiroshima, Sasayama, Atsumi, Kanto, Tohoku and Intermediate races) from the analysis of the geographic variations in the pattern of ventral markings and body proportions. Later, Mertens [25] gave a name *Triturus* (*=Cynops*) *pyrrhogaster sasayamae* for "Sasayama race" and the other "races" have remained unnamed and included in a single subspecies *C. p. pyrrhogaster*. Our biochemical study contained specimens of two "local races" belonging to different subspecies ("Sasayama race"=*C. p. sasayama*, distributed in northern Kinki and eastern Chugoku Districts (populations 1–8), and parts of "Hiroshima race" =*C. p. pyrrhogaster*, distributed in Kyushu, Shikoku and western Chugoku Districts (populations 9–23)). In the present study, *C. pyrrhogaster* showed significant heterogeneity in electromorph frequencies at all polymorphic loci (Table 4) and it is clear that local populations are genetically well isolated and diverged. Especially, southernmost Kyushu populations (populations 21–23) showed a high level of genetic differentiation from others, and consequently populations of Hiroshima race (i.e. *C. p. pyrrhogaster*) did not form a single group (Fig. 2). This unexpected result indicates that the widely ranging "Hiroshima race" contains at least two distinct groups. By contrast, the "Sasayama race" was found to form a single group.

Our data offer an interesting perspective not only into taxonomic problems but also into genetic divergence in *C. pyrrhogaster*. We are currently conducting an extensive sampling throughout the range of this species and additional electrophoretic studies are in progress.

The molecular clock hypothesis has been applied to date divergence events of taxa [26, 27]. Since molecular clocks need calibrations before applied, some calibrations were estimated for electrophoretic clock, which is a kind of molecular clocks, from different sources. Nei and Roychoudhury [11] originally suggested a calibration of 1D=5 MY irrespective of animal groups. Later, Maxson and Maxson [10] calibrated the electrophoretic clock at 1D=14 MY in plethodontid salamanders based on the correlation with the albumin clock, and the value has generally been used [28, 29]. Calibration could be obtained if geological information of the time of isolation between two populations is available. Because newts cannot cross over the sea, a strait must be a sufficient barrier for gene flow between populations. The formation of the straits between the Japanese main islands and the Ryukyu Archipelago assuredly marked the cessation of gene exchange between populations separated by the sea. The formation of the strait between the Amami and Okinawa Groups also must have prevented newts from gene exchange between isolated populations.

Kizaki and Oshiro [30, 31] estimated that the strait between the mainlands and the Ryukyu Archipelago south of Amami Group was formed about 8 MYBP during late Miocene times and that the strait between the Amami and Okinawa Groups was formed between 1 and 1.5 MYBP during middle Pleistocene times. If the mean D value between *C. ensicauda* and *C. pyrrhogaster* (0.356) corresponds to 8 MY, a calibration of 1D= 22 MY is obtained. On the other hand, when the mean D value between populations of the Amami and Okinawa Groups (0.078) is compared to the duration between 1 and 1.5 MY, the electrophoretic clock is calibrated at 1D=13–19 MY. Since both genetic and geological estimates themselves may contain a considerable amount of errors, the difference in these two estimates may not be so great. At least, calibrations of electrophoretic clock in Japanese *Cynops* are regarded as far greater than Nei's original estimate (1D=5 MY) and are more similar to the calibration used in plethodontid salamanders (1D=14 MY). This result seems to suggest the presence of limited range of calibration which is specific to the order Caudata.

REFERENCES

1 Inger, R. F. (1947) Preliminary survey of the amphibians of the Riukiu Islands. Fieldiana : Zool., **32**: 295–352.

2 Sawada, S. (1963) Studies on the local races of the Japanese newt, *Triturus pyrrhogaster* Boie. I. Morphological characters. J. Sci. Hiroshima Univ., Ser. B, 1, **21**: 135–165.

3 Kawamura, T. (1950) Studies on hybridization in amphibians. III. Reciprocal hybrids between *Triturus pyrrhogaster* (Boie) and *Triturus ensicauda* (Hallowell). J. Sci. Hiroshima Univ., Ser. B, 1, **11**: 71–79.

4 Nakamura, K. and Uéno, S.-I. (1963) Japanese Reptiles and Amphibians in Colour, Hoikusha, Osaka. (In Japanese)

5 Oyama, J. and Nakamura, D. (1939) Über Artbastarde *Triturus pyrrhogaster* (Boie) ♀ und *T. ensicauda* (Hallowell) ♂. Jpn. J. Genet., **15**: 78–79. (In Japanese)

6 Hedgecock, D. and Ayala, F. J. (1974) Evolutionary divergence in the genus *Taricha* (Salamandridae). Copeia, **1974**: 738–747.

7 Kalezić, M. L. (1984) Evolutionary divergences in the smooth newt, *Triturus vulgaris* (Urodela, Salamandridae) : electrophoretic evidence. Amphibia-Reptilia, **5**: 221–230.

8 Ragghianti, M. and Wake, D. B. (1986) Genic variation and its evolutionary implications in the Italian newt, *Triturus italicus*. Herpetologica, **42**:

206–214.

9   Tabachnich, W. J. (1977) Geographic variation of five biochemical polymorphisms in *Notophthalmus viridescens*. J. Hered., **68**: 117–122.

10 Maxson, L. R. and Maxson, R. D. (1979) Comparative albumin and biochemical evolution in plethodontid salamanders. Evolution, **33**: 1057–1062.

11 Nei, M. and Roychoudhury, A. K. (1974) Genic variation within and between the three major races of man, caucasoids, negroids, and mongoloids. Am. J. Hum. Genet., **26**: 421–443.

12 Ayala, F. J., Powell, J. R., Tracey, M. L., Mourão, C. A., and Pérez-Salas, S. (1972) Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics, **70**: 113–139.

13 Delorenzo, R. L. and Ruddle, F. H. (1970) Glutamate oxaloacetate transaminase (GOT) genetics in *Mus musculus*; linkage, polymorphism, and phenotypes of the GOT-2 and GOT-1 loci. Biochem. Genet., **4**: 259–274.

14 Sato, C. (1982) The variation of proteins and enzymes in blood. In "Methodology of Human Genetics". Ed. by E. Matsunaga, Kyoritsu-Shuppan, Tokyo, pp. 114–155. (In Japanese)

15 Shaw, C. R. and Prasad, R. (1970) Starch gel electrophoresis of enzymes — a compilation of recipes. Biochem. Genet., **4**: 297–320.

16 Selander, R. K., Smith, M. H., Yang, S. Y., Johnson, W. E. and Gentry, J. B. (1971) Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet. 6. Univ. Texas Publ., **7103**: 49–90.

17 Nomenclature Committee of the International Union of Biochemistry. (1984) Enzyme Nomenclature 1984. Academic Press, NY.

18 Murphy, R. W. and Crabtree, C. B. (1985) Evolutionary aspects of isozyme patterns, number of loci, and tissue-specific gene expression in the prairie rattlesnake, *Crotalus viridis viridis*. Herpetologica, **41**: 451–470

19 Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

20 Sneath, P. H. A. and Sokal, R. R. (1973) Numerical Taxonomy. W. H. Freeman Co., San Francisco.

21 Workman, P. L. and Niswander, J. D. (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Hum. Genet., **22**: 24–29.

22 Kalezić, M. L. and Hedgecock, D. (1980) Genetic variation and differentiation of three common European newts (*Triturus*) in Yugoslavia. Br. J. Herpetol., **6**: 49–57.

23 Koba, K. (1962) Studies on the Snakes of the Genus *Trimeresurus* of the Amami and Tokara Islands. Japan, Japan Society for the Promotion of Science, Tokyo. (In Japanese)

24 Hedgecock, D. (1976) Genetic variation in two widespread species of salamanders, *Taricha granulosa* and *Taricha torosa*. Biochem. Genet., **14**: 561–576.

25 Mertens, V. R. (1969) Über die Rassen des Feuerbauchmolches (*Triturus pyrrhogaster*) und ihre wissenschaftlichen Namen. Aquar.-Terrar.-Z., **22**: 114–117.

26 Sarich, V. M. and Wilson, A. C. (1967) Immunological time scale for hominid evolution. Science, **158**: 1200–1203.

27 Thorpe, J. P. (1982) The molecular clock hypothesis : biochemical evolution, genetic differentiation and systematics. Ann. Rev. Ecol. Syst., **13**: 139–168.

28 Larson, A. (1983) A molecular phylogenetic perspective on the origins of a lowland tropical salamander fauna. I. Phylogenetic inferences from protein comparisons. Herpetologica, **39**: 85–99.

29 Larson, A., Wake, D. B., Maxson, L. R. and Highton, R. (1981) A molecular phylogenetic perspective on the origins of morphological novelties in the salamanders of the tribe Plethodontini (Amphibia, Plethodontidae). Evolution, **35**: 405–422.

30 Kizaki, K. and Oshiro, I. (1977) Paleogeography of the Ryukyu Islands. Marine Sciences Monthly, **9**: 542–549. (In Japanese with English summary)

31 Kizaki, K. and Oshiro, I. (1980) The Origin of the Ryukyu Islands. In "Natural History of Ryukyu". Ed. by K. Kizaki, Tsukiji-Shokan, Tokyo, pp. 8–37. (In Japanese)

[COMMUNICATION]

Effects of Adult Male Cohabitation on Precocious Puberty in Early Weaning Female Guinea Pigs

Tadashi Kosaka, Masaki Obata, Toru R. Saito
and Kazuaki W. Takahashi[1]

Toxicology Division, Institute of Environmental Toxicology, Suzuki-cho 2-772,
Kodaira-shi, Tokyo 187, and [1]Department of Laboratory Animal Science,
Nippon Veterinary & Zootechnical College, Kyonan-cho 1-7-1,
Musashino-shi, Tokyo 180, Japan

ABSTRACT—The effects of adult male cohabitation with infant female guinea pigs weaned at 2 days or 4 days of age on the onset of puberty were investigated. The females weaned at 4 days had no growth abnormalities, while 2 of 20 females weaned at 2 days died due to developmental abnormalities. Cohabitation with an adult male significantly advanced the day of vaginal opening in the females weaned at 4 days. During the first vaginal opening, 4 of 9 and 7 of 10 females with male cohabitants in the groups weaned at 2 and 4 days old, respectively, copulated, became pregnant and delivered normally. These results indicated that advancement of female puberty was produced by continuous exposure to an adult male even in infant female guinea pigs weaned at 4 days.

INTRODUCTION

Social stimuli that operate primarily through olfactory mechanisms, including presence of males, influence reproductive biology in mice [1–4]. Similar sensory mechanisms are undoubtedly involved in modifying animal reproduction. Previous reports have shown that the presence of an adult male accelerates the sexual maturation of female mice [1, 2, 5–9], rats [3], pigs [10] and human beings [11]. In guinea pigs, our previous work [12] indicated that a similar advancement in puberty was caused by adult male cohabitation from the day of normal weaning (1 or 2 weeks of

age). It is known that newborn babies of guinea pigs are well-developed compared with other rodents, i.e., the babies have a fully developed coat, walk immediately and eat food; their teeth are useful and their eyes are open. Thus, it seems possible that social stimuli can play a useful role in infant guinea pigs weaned earlier than normal.

The present study, therefore, was designed to investigate the influence of continuous exposure to an adult male on the onset of female sexual maturation in infant female guinea pigs weaned early.

MATERIALS AND METHODS

Albino guinea pigs of the Hartley strain were used. They were kept in a controlled semi-barrier system room with a temperature of $22 \pm 2°C$, a photoperiod of 14 hr light/10 hr dark (lights on from 05:00 to 19:00 hr) and ventilation 12 times an hour. The females were housed individually, either alone or with an adult male cohabitant in an aluminum cage ($260W \times 400D \times 200H$ mm), and given commercial pellets (GB–1, Funabashi Farm Inc.) and tap water *ad libitum*.

Infant female guinea pigs were weaned at the age of 2 days (group A) and 4 days (group B). Each group was then divided into two subgroups. One subgroup of females was continuously exposed to an adult male (5 to 12 months old) with proven fertility from the time of weaning until the

end of the first vaginal opening by placing the male directly in the cage containing the female; the other subgroup of females was housed alone. Vaginal closure membranes were examined once a day until the end of the second vaginal opening. Vaginal opening was determined to be positive when the vaginal membrane was ruptured. Each animal was weighed at the first vaginal opening. In the females mating with males during the first vaginal opening, the gestation period and litter size were recorded. The day when spermatozoa were found in vaginal smears was designated as day 0 of pregnancy. Student's *t* test was used to determine the level of significance.

RESULTS AND DISCUSSION

The results are summarized in Table 1. With regard to growth after weaning, 2 of 20 females weaned at 2 days of age (group A) died 2 days after weaning due to developmental abnormalities. In the group weaned at 4 days (group B), the mean age at the first vaginal opening in the females housed with a male was significantly younger compared with females housed alone. In females weaned at 2 days (group A), however, the females with a male had a slight but not significant advancement of the first vaginal opening over females housed alone. The mean body weight at the first vaginal opening in females with a male was significantly less than that of females without a

male in both groups A and B (group A, $P<0.05$; group B, $P<0.01$). Mean duration of the first vaginal opening did not differ between the subgroups of group A, but it was significantly reduced in females together with a male in group B ($P<0.05$) when compared with females housed alone. During the first vaginal opening, 11 out of 19 females housed with a male (4 of 9 females in group A and 7 of 10 in group B) copulated and became pregnant. The mean gestation period in group A, 72.8 ± 1.8 days, was longer than that in group B, 69.3 ± 1.8 days ($P<0.05$). There was no significant difference between the mean litter size in the groups weaned at 2 and 4 days, 2.3 ± 1.0 and 2.0 ± 0.8, respectively.

Concerning the relationship between the time of vaginal opening and body growth, it is unclear whether or not precocious puberty caused by the presence of males is controlled by body growth. In this study, acceleration of the first vaginal opening due to male cohabitation was induced without the female reaching a body weight, as high as that of females housed alone at the time of first vaginal opening. Damon *et al.* [13] indicated that physical growth and sexual maturation might be a separate process. The presence of the male, therefore, which is an olfactory stimuli, and not physical growth, may lead to the hastening of puberty.

Our previous work [12] reported that female guinea pigs with an adult male cohabitant from the time of normal weaning (1 or 2 weeks old)

TABLE 1. Effects of an adult male cohabitant on the age at vaginal opening, the opening duration and body weight at the first vaginal opening in female guinea pigs

Age at weaning (Group)	Cohabitant	No. of animals examined	Age at first vaginal opening (days)	Duration of first vaginal opening (days)	Body weight at first vaginal opening (g)
2 days (group A)	None	9	36.9 ± 3.6	5.1 ± 0.6	312.3 ± 11.4
	Male	9	33.2 ± 5.8	6.6 ± 1.0	$269.4\pm14.1^*$
4 days (group B)	None	10	37.3 ± 1.8	7.4 ± 0.7	306.7 ± 6.2
	Male	10	$28.1\pm2.5^{**}$	$4.8\pm0.7^*$	$259.0\pm12.0^{**}$

All data represent mean $\pm$ S.E.
*, **: Significantly different from the non-cohabitant subgroup of each group at the 5 and 1% level of probability, respectively.

accelerated puberty. In this paper, the presence of males resulted in precocious puberty characterized by advancement of vaginal opening in infant female guinea pigs weaned earlier (group B) than normal. In group A, the time of first vaginal opening in females with and without males ranged from 16 to 66 and from 29 to 64 days of age, respectively. In the females housed with a male, the age at the first vaginal opening was slightly but not significantly accelerated, that is, the ages at the first vaginal opening of 5 females (16, 19, 22, 25, 27 days of age) out of 9 were hastened, when compared with the age at the first vaginal opening (29 days) in the females housed alone. Precocious puberty caused by the presence of males might be not related to the exposure time. Takahashi *et al.* [14] reported that the shortening of the rat estrous cycle caused by the presence of males was observed in higher incidences when different adult male rats were frequently exposed to females. In this study, the exposure of a fresh male to the females of groups A and B was started at 2 and 4 days of age, respectively. If the critical age of sensitivity to the fresh stimuli of a male cohabitant existed around 2 days of age in female guinea pigs, the above-mentioned slight acceleration of puberty should be induced. However, during the first vaginal opening, 7 out of 10 females in the group weaned at 4-days-old copulated, became pregnant and delivered normally. These results, therefore, indicate that weaning at 4 days was the earliest time at which male cohabitation had an effect on the precocious sexual maturity in the females.

REFERENCES

1 Vandenbergh, J. G. (1967) Endocrinology, **81**: 345–349.
2 Vandenbergh, J. G. (1969) Endocrinology, **84**: 658–660.
3 Vandenbergh, J. G. (1976) J. Reprod. Fertil., **46**: 451–453.
4 Parkes, A. S. and Bruce, H. M. (1961) Science, **134**: 1049.
5 Eisen, E. J. (1975) J. Anim. Sci., **40**: 816–825.
6 Kennedy, J. M. and Brown, K. (1970) Dev. Psychobiol., **3**: 179–189.
7 Marsden, H. M. and Bronson, F. H. (1965) J. Endocrinol., **32**: 313–319.
8 Nobunaga, T., Nakamura, K. and Yoshida, Y. (1965) Japan. J. Anim. Reprod., **11**: 7–15.
9 Takahashi, K. W. (1971) Japan. J. Anim. Reprod., **17**: 47–54.
10 Brooks, P. H. and Cole, D. J. A. (1970) J. Reprod. Fertil., **23**: 435–440.
11 McClintock, M. K. (1971) Nature, **229**: 244–245.
12 Saito, T. R., Obata, M. and Takahashi, K. W. (1982) Japan. J. Anim. Reprod., **28**: 141–144.
13 Damon, A., Damon, S. T., Reid, R. B., and Valadian, I. (1969) Hum. Biol., **41**: 161.
14 Takahashi, K. W., Saito, T. R. and Amao H. (1986) Bull. Nippon Vet. Zootec. Coll., **35**: 124.